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Boiled or Filtered Coffee?

Effects of Coffee and Caffeine on Cholesterol, Fibrinogen and C-Reactive Protein

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Contents

Abstract

Caffeine is the most widely consumed psychostimulant drug in the world that mostly is consumed in the form of coffee. Whether caffeine and/or coffee consumption contribute to the development of cardiovascular disease (CVD), the single leading cause of death in the US, is unclear.

This article examines the effects of caffeine intake, both alone and via coffee consumption, on key blood markers of CVD risk: lipoproteins (cholesterol, triglycerides), fibrinogen (a biomarker of blood clotting) and C-reactive protein (CRP; a biomarker of inflammation). These blood markers and their role in the development of CVD are reviewed first. Studies examining caffeine and coffee effects on each of these blood markers are then presented. Next, biobehavioural moderators of the relationship between caffeine and/or coffee consumption and CVD are discussed, including genetics, sex and tobacco smoking.

The literature indicates a strong relationship between boiled, unfiltered coffee consumption and elevated cholesterol levels; however, there is a critical gap in the literature regarding the effects of coffee or caffeine consumption on fibrinogen or CRP, which is an independent predictor of CVD risk. Available studies are limited by small samples sizes, inclusion of only men (or few women) and unrepresented age or ethnic groups. Thiere is a critical need for controlled laboratory and epidemiological studies that include fibrinogen and CRP markers of CVD risk before conclusions can be drawn regarding the health effects of caffeine and/or coffee in a normal, healthy population of men and women.

1. Scope of the Problem

Caffeine is the most widely consumed psychostimulant drug in the world, with about 75% of its consumption taken in the form of coffee.[1] Other sources of caffeine include tea, caffeinated cola drinks and chocolate, as well as herbal supplements and prescription and over-the-counter diet drugs.[2] In the US, of those individuals who drink coffee, the average consumption is 3 cups per day.[2] Higher caffeine levels are consumed in Central and South America and Western Europe, compared with the US, and the highest level of consumption is found in Scandinavia, especially Finland and Sweden.[3]

A number of researchers have reported adverse effects of caffeine on different aspects of health, such as cardiovascular disease (CVD),[4] fibrocystic breast disease,[5,6] several types of cancer[1] and reproductive problems.[7-9] Since the 1900s, CVD has been the number one killer in the US every year except 1918. Indeed, nearly 2600 Americans die of CVD each day, an average of one death every 33 seconds. The purpose of this article is to review the current knowledge on the relationship between caffeine (coffee being its major source) and CVD risk. The importance of better understanding how caffeine and coffee can affect the development of CVD could have a tremendous impact on improving health outcomes. Not only is caffeine the most widely consumed psychoactive drug, but for the majority of caffeine consumers, exposure to this drug is effectively lifelong.[2] Level of exposure is a key factor in assessing the cumulative biobehavioural impact of any drug-induced effects. Considering current levels of caffeine use, even small effects, when accumulated across entire populations, may have very important health consequences.

CVD risk markers include elevated resting systolic and/or diastolic blood pressure, low density lipoproteins (LDL), triglycerides, blood clotting factors (e.g. fibrinogen) and the acute-phase protein C-reactive protein (CRP). To the extent that changes in these markers are associated with exposure to caffeine, usually in the form of coffee, then a biological mechanism(s) that underlies the relationship between caffeine consumption and CVD can be better understood. The relationship between acute caffeine consumption and elevated blood pressure levels has been well established, along with vascular mechanisms for this effect.[10] What is less understood is the role that caffeine exposure and coffee intake play in influencing the development of CVD via blood markers of CVD.

This article outlines the literature that examines the relationship between caffeine intake, primarily via coffee consumption, and primary blood makers of CVD risk. More specifically, this article focuses on four blood markers that have been shown to be related to the development of CVD, namely, blood lipids (which include cholesterol and triglycerides), fibrinogen and CRP. With respect to these blood markers, blood lipids (especially cholesterol) and their relationship to coffee has been the most studied, while very few studies have examined the effects of either coffee or caffeine by itself on fibrinogen and CRP. This article also highlights key methodological problems (e.g. controlling for diet, sample size, tobacco use) and gaps (e.g. effects of caffeine on these markers among women) that still exist in our understanding. Coffee is the main source of caffeine; however, very little work has been done examining the direct effects of caffeine on these blood markers in humans. Therefore, this article focuses primarily on coffee, although caffeine-specific studies are included when they are available.

2. Blood Markers of Cardiovascular Health

2.1 Cholesterol

Cholesterol, a waxy, fatty substance found in the bloodstream is necessary for the normal functioning of various body systems, such as the production of cell membranes and a number of hormones.[11] A link between high levels of total cholesterol and a higher incidence of CVD was first reported in 1955.[12] Although a high level of total serum cholesterol appears to be associated with the development of cardiac disease,[13,14] it is important to examine the two main types of cholesterol, high-density lipoproteins (HDL) and LDL, which are better predictors of CVD than is total serum cholesterol alone. HDL-cholesterol (HDL-C) is known as the beneficial kind because it seems to protect against CVD, while LDL-cholesterol (LDL-C) is considered to be the detrimental kind as it appears to increase one's risk of developing CVD[13-17] by transporting cholesterol from the liver to the tissues of the body. In contrast, HDL carries cholesterol molecules away from arteries and back to the liver, where these molecules are metabolised and excreted. It is also believed that HDL removes excess cholesterol from plaques that have already formed in arteries, therefore slowing down the build-up process that leads to atherosclerosis and the development of CVD.[11]

There are some important caveats to keep in mind when reviewing the literature on changes in blood cholesterol levels. First, triglycerides are typically associated with lower levels of HDL-C.[18-20] Triglycerides are also a form of fat, which primarily come from dietary fat, but also are endogenously produced.[11] Second, dietary fat intake, age, body mass index (BMI; weight [kg]/height [m^2]), and cigarette smoking have been found to be positively associated with total cholesterol levels and LDL-C levels.[21] In contrast, physical activity and moderate doses of alcohol have been found to be negatively associated with serum total and LDL-C levels.[22] Therefore, these variables need to be controlled for or acknowledged when interpreting the effects of caffeine on these blood markers.

2.2 Fibrinogen

Fibrinogen, a clotting protein produced in the liver, has also been associated with CVD.[11,23-29] This soluble acute-phase protein circulates in the blood and provides the materials from which the insoluble fibrin clot is formed during blood coagulation. Fibrinogen, therefore, helps to stop bleeding by helping blood clots to form in a process that involves the breakdown of fibrinogen by thrombin into short fragments of fibrin, which activate the release of factor XIII. Factor XIII, in turn, weaves the fibrin segments together, closing off the injured blood vessel walls; blood platelets then attach to the fibrin segments and clump together to form blood clots and stop bleeding.[30] Fibrinogen levels increase in response to a number of factors, such as infection and other short-term inflammatory stressors, such as cigarette smoking. Elevated fibrinogen levels are also seen with advancing age, obesity, diabetes mellitus, physical inactivity and high levels of blood LDL and total cholesterol.[28]

High plasma fibrinogen levels in adulthood have been associated with a statistically significant increase in the risk of CVD and stroke. For example, prospective studies in both men [23,24,29-31] and women[24] have shown that a single fibrinogen measurement predicts fatal and non-fatal cardiovascular events as much as 16 years later.[30] Along the same lines, other investigators have found that fibrinogen levels were higher among participants with CVD compared with those without CVD.[27,28] At this point, researchers are not entirely sure as to whether a rise in fibrinogen is a cause or a consequence of CVD. Fibrinogen may promote atherosclerotic changes and thrombosis through increases shown *in vitro* on platelet aggregability and blood viscosity.[31] This increased aggregation and change in blood viscosity increases the risk of stroke and heart attack. As a result, this process seems indicative of a causal role for fibrinogen in CVD. An alternative view is that the prospective association between fibrinogen and CVD may be a consequence, rather than a cause, of the disease process, perhaps due to an inflammatory response to progressive endothelial damage.[23,24] Both of these perspectives, which are not mutually exclusive, support the hypothesis that fibrinogen may play a role in the development of CVD.[32]

2.3 C-Reactive Protein (CRP)

CRP is an acute-phase protein that increases in response to system inflammation and has received a lot of recent scientific attention due to its correlation with increased risk for CVD beyond that of LDL-C levels.[33-36] What makes this marker an exciting advancement in our understanding of the development of CVD is that it appears to make an independent contribution to CVD that goes beyond currently available disease risk markers such as fibrinogen, LDL-C and arterial wall thickness,[37,38] although an alternative view has been proposed.[39] For example, CRP elevations independently increase the risk of stroke, above the level of risk that would be predicted by the increased thickness of carotid artery walls (typically an indicator of atherosclerotic plaque in the arteries leading to the brain).[38] In addition, individuals with elevated CRP levels do not receive the same cardiovascular benefits of cholesterol reduction while on a low-fat, low-cholesterol diet as do those with lower CRP levels.[40] Furthermore, elevated CRP levels, combined with high blood pressure levels, appears to place women at a significantly greater risk for heart attacks and strokes compared with women with hypertension who do not have elevated CRP levels.[33] At this time, however, no studies have described a mechanism by which CRP may lead to CVD.

2.4 Summary

Taken together, the literature suggests that lipoproteins (e.g. LDL-C), fibrinogen and CRP are important blood markers of CVD risk that are sensitive to biobehavioural influences such as age, bodyweight, sex and diet, including, perhaps, caffeine and coffee intake. To the extent that changes in these markers are associated with different levels of caffeine intake, a better understanding of the mechanisms that underlie the effects of caffeine intake on the development of CVD could be developed. The rest of this article will focus on the effects of caffeine and coffee consumption on these blood markers.

3. Caffeine, Cholesterol and Triglycerides

3.1 Early Findings

Early laboratory-based findings on the relationship between caffeine and its potential to elevate blood lipids (i.e. cholesterol and triglycerides) are inconsistent, with some studies reporting a significant rise in cholesterol levels following ingestion of caffeine through coffee in rats and rabbits,[41] and also in humans,[42] and others showed no effect in rhesus monkeys[43] or humans.[44] Eventually, however, epidemiological studies began to report a strong positive association between coffee consumption and elevated blood lipid levels, which attracted much attention to this area of study due to the known positive connection between serum total cholesterol and LDL-C and the development of CVD. The major findings of these epidemiological studies examining the relationship between consumption of coffee and its effect on blood lipids are discussed in section 3.2, as well as key methodological flaws.

3.2 Epidemiological Studies Examining the Relationship Between Coffee and Blood Lipids

One of the first epidemiological studies to report a strong association between consumption of coffee and blood lipid levels was the Tromso Heart Study.[45] This study was conducted in Tromso, Norway, and included 7213 women and 7368 men between the ages of 20 and 54 years, following a screening in 1979–80. The major finding of this study was that coffee consumption was strongly positively associated with levels of total cholesterol and triglycerides in both men and women, and was negatively associated with HDL-C levels in women. This relationship remained very strong and statistically significant ($p < 0.0001$) even after adjustment for age, BMI, physical activity, alcohol consumption and cigarette smoking.[45] Interestingly, the predominant type of coffee consumed in the Tromso region is prepared by boiling, while other European regions drink predominantly filtered and French-press coffee. This difference in preparation methods led the investigators to question whether the brewing method could be the explanation to the rise in blood lipids.

As a follow-up to the initial Tromso study,[45] the investigators designed a study intended to assess the effects of coffee consumption and coffee brewing methods on serum cholesterol concentrations in men. Thirty-three men diagnosed with hypercholesterolaemia (i.e. elevated cholesterol levels) were randomised into one of the three following conditions: (i) participants were told to continue their usual coffee intake; (ii) participants were told to stop drinking coffee; or (iii) participants were told to stop drinking coffee for 5 weeks, followed by consumption of either boiled or filtered coffee. Cholesterol concentration significantly dropped in all of the participants abstaining from coffee for 5 weeks, and continued to drop in those who abstained for 10 weeks, compared with participants who continued to drink coffee. Cholesterol concentrations significantly rose in those participants who began drinking boiled coffee but remained the same in those drinking filtered coffee following abstinence.

Unfortunately, this study did not include women, even though a negative association between boiled coffee and HDL-C was only found for women in the initial study. As a result, the results only apply to men, which leaves out important information regarding what the effects of coffee consumption and brewing method may be for women. This information is especially important in light of their early findings indicating that women may be at higher risk for the development of CVD due to a decrease in HDL-C levels in response to coffee. This study also did not include elderly individuals, who might display an even more pronounced increase in serum lipid levels,[46,47] and are more prone to the development of CVD than a younger population. Although this study seemed to provide some evidence that brewing method may be an explanation for the cholesterol-raising effects of coffee, it was still early to say conclusively whether this was the case, because this was the first study to examine this relationship. Brewing methods will be further explored in section 3.3, examining the rest of the experimental evidence of a relationship between coffee and blood lipids.

In the early 1990s, other epidemiological studies began to emerge such as the Heidelberg-Michelstadt-Berlin Study[48] and the Olivetti Heart Study.[49,50] These studies started to account for a number of variables that had not been previously examined, such as age, sex, alcohol consumption and cigarette smoking. Kohlmeier et al.[48] in the Heidelberg-Michelstadt-Berlin Study, investigated the relationship between coffee consumption and serum cholesterol levels in a population of 395 young (aged 18–24 years) and 385 elderly (65–74 years) German men and women. Some of the methodological highlights of this study were that the investigators separately analysed the data from men and women in order to determine whether any sex differences existed, as well controlling for: BMI; total calorie intake; fat, fish, tea and milk intake; physical activity; alcohol consumption; smoking; and the use of oral contraceptives. Elevated levels of total serum and LDL-C were positively correlated with increased coffee intake for the group of young men only. This study showed no trend between the consumption of coffee and rises in total or LDL-C in the elderly.

The next epidemiological study to report a positive relationship between coffee consumption and serum lipids was the Olivetti Heart Study,[49] which was carried out at the Olivetti factory in Naples, Italy. After 12 years of follow-up, the investigators obtained questionnaires from 942 men and 48 women. This study took into account important variables such as BMI, smoking and age. Due to the very small number of women, they were excluded from statistical analyses. In this study, 900 men were included in the statistical analyses and it was revealed that increased coffee consumption (measured in cups per day) was associated with lower levels of HDL-C, as well as positively related to serum triglyceride levels. Interestingly, however, after accounting for tobacco smoking status, a significant positive linear trend between coffee consumption and total serum cholesterol only was observed in smokers. No significant trend was found among smokers for LDL-C and triglycerides.[49] Although this study did not examine the effects of coffee consumption in women and did not include other important variables such as physical activity and dietary intake, as well as coffee preparation method, it does show that cigarette smoking may change the relationship between coffee consumption and cholesterol levels. Therefore, investigators should further examine this variable.

Taken together, the findings of these epidemiological studies illustrate a positive relationship between coffee consumption and serum cholesterol levels. With the exception of Kohlmeier et al.[48]

who did not report a significant increase in total serum and LDL-C in the elderly, but did find a significant relationship among young participants, all of the other investigators[45,49-51] found a significant increase in serum total cholesterol in response to coffee, in addition to a significant increase in LDL,[48] and a significant decrease in HDL-C.[45,49] Interestingly, Jossa et al.[49] found that after accounting for smoking status, a significant increase in serum total cholesterol was only seen in smokers. Therefore, it appears that cigarette smoking increases the risk of the negative effects of coffee on serum total cholesterol. Other studies did not account for tobacco smoking, so this variable should be further examined in future studies. Although not all of the studies included important variables such as the inclusion of women, careful monitoring of dietary intake, smoking habits, BMI and alcohol consumption, the common variable in all of the studies is a correlation between coffee consumption and higher levels of serum total cholesterol. At this time, no epidemiological studies have investigated the effects of coffee or caffeine on CRP or fibrinogen.

Despite the fact that these epidemiological studies do not elucidate the potential mechanisms through which coffee may be leading to rises in cholesterol levels and therefore an increased risk for CVD, these studies do set the ground for true experimental studies, where different hypotheses can be explored such as whether the preparation method may be the missing link between coffee consumption and rises in serum cholesterol. Throughout section 3.3, a review of experimental studies will be presented and potential mechanisms of action for the coffee/cholesterol connection will be discussed.

3.3 Potential Mechanisms that Contribute to the Relationship Between Coffee Consumption and Cholesterol Levels: Human Studies

Because epidemiological findings brought about the question of whether the brewing method played a significant role in the coffee/cholesterol connection, experimental studies were designed to test that hypothesis and also to determine a mechanism that would explain the connection between coffee intake and a rise in cholesterol levels. This section will describe the research that has led to our current understanding of how coffee consumption may lead to an increase in cholesterol.

3.3.1 Boiled versus Filtered Coffee Effects on Lipids

Although epidemiological studies led to the questioning of whether preparation method could be the cause leading to an increase in cholesterol following exposure to coffee, only carefully controlled experimental designs would truly answer this question. These types of studies began to emerge in the late 1980s and the findings indeed seemed to support the hypothesis that preparation method may be a factor explaining the coffee/cholesterol connection.[52-56] The reason for this is that coffee contains a lipid component that may be responsible for its cholesterol-raising effects. Unfiltered coffee brews contain approximately 1–2g of lipids per litre, while lipid levels found in filtered coffee are nearly negligible.[57]

In 1989, Bak and Grobbee[52] conducted a study examining the effects of filtered and boiled coffee on total serum cholesterol and cholesterol fractions. This 12-week randomised trial included 107 young adult male and female participants with normal serum cholesterol levels. After a 3-week run-in period during which they all consumed filtered coffee, the participants were randomly assigned to one of three groups receiving 4–6 cups of boiled coffee a day, 4–6 cups of filtered coffee a day, or no coffee, for a period of 9 weeks. Compared with baseline serum total cholesterol levels, there was a 10% increase in cholesterol levels among participants who consumed boiled coffee for 9 weeks. There was no significant difference in the change of LDL-C levels between the filtered-coffee group and the group that drank no coffee.[52] The findings of this study indicate that boiled coffee consumption increases serum total cholesterol levels, whereas filtered coffee does not. These findings suggest that the lipid component of boiled coffee may be the causative agent responsible for the cholesterol-raising effects of coffee.

Following these findings, other investigators sought to test the hypothesis of whether the lipid part of boiled coffee was the responsible component for the cholesterol-raising effects seen with coffee consumption.[53] Ten volunteers (five men, five women) consumed a lipid-enriched fraction from boiled coffee for 6 weeks. Serum total cholesterol rose in every subject, mainly due to LDL-C (29% increase), as well as a 55% average triglyceride increase. HDL-C was unchanged. After supplementation ended, lipid levels returned to baseline.[53] Therefore, this study shows that boiled coffee contains a lipid that powerfully raises serum cholesterol. Although the rises seen in total and LDL-C levels, as well as triglycerides, were reversible, it is important to keep in mind that for the vast majority of coffee consumers, the exposure to coffee is life long. Furthermore, an increase of 29% in LDL-C, as well as 55% rise in triglycerides can be of clinical significance. For example, such an increase may push someone with borderline high levels into a category where they are considered to be abnormally high and may require medical attention.

van Dusseldorp et al.[58] investigated whether boiled coffee that is filtered still results in elevated cholesterol levels by examining cholesterol changes among participants who drank boiled, filtered coffee to those who drank boiled, unfiltered coffee or no coffee at all. Serum cholesterol increased only among participants who drank boiled, unfiltered coffee, a finding that was confirmed in subsequent studies with participants who had elevated blood lipid profiles (i.e. hyperlipidaemia).[55]

Although studies examining the lipid component of boiled coffee appeared to consistently find that the lipid component was the responsible variable for significant rises in cholesterol and that filtering would remove (or at least decrease it enough so that rises in cholesterol would not be clinically significant) the cholesterol-raising component in coffee, a small number of studies suggested that filtered coffee may also increase cholesterol levels, and began to cast some doubt into what appeared to be a fairly clear picture. Fried et al.[59] designed a randomised controlled trial with an 8-week washout period followed by an 8-week intervention period during which men were randomly assigned to drink one of the following filtered coffee conditions: (i) 720mL of caffeinated coffee; (ii) 360mL of caffeinated coffee; (iii) 720mL of decaffeinated coffee; or (iv) no coffee at all. All coffee consumed in this study was filtered. Participants were 100 healthy men. A significant increase in plasma total cholesterol (0.25 mmol/L, $p = 0.02$), LDL-C (0.15 mmol/L, $p = 0.17$), and HDL-C (0.09 mmol/L, $p = 0.12$) was observed after the consumption of 5 cups of filtered coffee for 8 weeks.[59] Christensen et al.[60] also observed a decrease of 0.28 mmol/L in plasma total cholesterol levels following cessation of approximately 4 cups per day of filtered coffee for 6 weeks in 191 healthy, nonsmoking, coffee-drinking volunteers aged 24–69 years.

To assess the effects of intake and abstention of filtered coffee on blood lipids, Strandhagen and Thelle[56] designed a prospective, controlled study involving 121 healthy, nonsmoking men and women aged 29–65 years. The study consisted of four consecutive trial periods: the first and third periods were 3 weeks of total coffee abstention, the second and fourth periods consisted of 4 weeks with participants consuming 600mL of filter-brewed coffee per day. Coffee abstention for 3 weeks decreased total serum cholesterol by 0.22–0.36 mmol/L (approximately 9–14 mg/dL). A volume of 600mL (about 4 cups) of filtered coffee per day during 4 weeks raised total serum cholesterol by 0.15–0.25 mmol/L (approximately 6–10 mg/dL). Although these numbers do not appear to be very high, even a change of 0.15–0.25 mmol/L may increase one's risk for developing CVD. For example, an increase of 1 mmol/L total cholesterol in those who have a total cholesterol level near 5.2 mmol/L places these individuals at twice the risk for CVD as those who have total cholesterol levels that are <200 mg/dL.

Taken together, these findings indicate that although the cholesterol-raising effects brought about by the consumption of filtered coffee may not be as strong as that of the boiled coffee, it is important not to discard the possibility that filtered coffee may also play a small but important role in explaining the cholesterol-raising effects of coffee. Although these findings do not appear to be clinically significant, at least in healthy populations, these findings may be of particular importance for those individuals already experiencing hyperlipidaemia. While studies have not yet been carried out to test this hypothesis, previous studies do suggest that these small changes may play a greater and more significant role in a hyperlipidaemic population.[55] Therefore, continuation of research in this area, especially including those with high levels of blood lipids, continues to be necessary.

3.3.2 Caffeine versus Coffee Effects on Lipids

Investigators then began to question whether the cholesterol-raising factor found more predominantly in boiled coffee and less predominantly in filtered coffee was related to caffeine content. In a 12-week double-blind trial, van Dusseldorp et al.[54] examined the effect of decaffeinated versus regular coffee on the serum lipid levels of 45 healthy volunteers (23 women and 22 men aged 25–45 years) with a habitual intake of 4–6 cups of regular coffee per day. Participants received 5 cups of regular coffee each day for 6 weeks and 5 cups of decaffeinated coffee for the next 6 weeks, or vice versa. Their background diet was kept constant and was low in caffeine. Differences between the effects of decaffeinated and regular coffee on total serum cholesterol, HDL-C and serum triglycerides were essentially zero, leading to the conclusion that, in healthy adults, replacement of regular coffee by decaffeinated coffee does not affect serum cholesterol and lipoproteins and that caffeine does not appear to play a role in the cholesterol-raising effects of coffee.[54]

Other trials studying caffeine effects on lipid levels have reported similar results. Bak and Grobbee[61] used a double-blind, randomised trial with two parallel groups in 69 young, male and female healthy participants. After a 3-week run-in period, participants were randomly assigned to one of two groups receiving either 4–6, 140mL cups of filtered decaffeinated coffee per day and an equal number of pills containing 75mg caffeine or 4–6 140mL cups of filtered decaffeinated coffee per day and an equal number of placebo pills for 9 weeks. In both groups, caffeine intake from other sources was not allowed. At the end of the study, abstinence from caffeine for a period of 9 weeks showed no effect on either serum lipids (total serum cholesterol, LDL-C and triglycerides) or blood pressure, and caffeine consumers did not show significantly elevated blood lipids or blood pressure.[61] Other investigators examining the effects of two kinds of decaffeinated coffee on serum lipid profiles also describe similar findings. Wahrburg et al.[62] found that consumption of two different types of decaffeinated coffee did not lead to any significant changes in serum total and LDL-C or triglycerides compared with filtered caffeinated coffee in a sample of 119 healthy students (60 men, 59 women). All of the participants in this study consumed 750–1000mL of caffeinated filtered coffee per day for a 2-week wash-in period, followed by a 6-week test period where one group continued drinking the caffeinated coffee, while the two other groups consumed different kinds of decaffeinated coffee.[62]

The literature examining the effects of caffeine on serum blood lipids consistently demonstrates that caffeine does not appear to play a role in the cholesterol-raising effects of coffee. However, to our knowledge, only one study[61] has specifically studied caffeine by itself (in the form of caffeine pills) while other studies have studied the effects of caffeine by comparing caffeinated and decaffeinated coffee. It is important to note that, to date, all of the studies examining the effects of caffeine on blood lipids have used healthy volunteers. Based on previous research by Hryniewiecki et al.,[55] it is possible that those populations with hyperlipidaemia (i.e. high blood lipid levels) may respond differently. For example, those with hyperlipidaemia may show a greater response to the effects of caffeine on blood lipids compared with those with normal lipid levels. This area of research should be further explored with more studies that include caffeine consumption alone.

3.4 Potential Mechanisms that Contribute to the Relationship Between Coffee Consumption and Cholesterol Levels: Animal Studies

Animal studies have examined the relationship between coffee and cholesterol in gerbils, hamsters, monkeys and rats. Mensink et al.[63] designed a study in which three groups of 20 male gerbils and three groups of six male hamsters were fed either a control diet or a control diet supplemented with either freeze-dried boiled coffee or freeze-dried filtered coffee. The hamsters were 4–6 weeks old. At the end of this study, it was reported that following the 5-week feeding period serum cholesterol levels were not different in either species fed the different diets.[63] Although these results suggest that perhaps these animal species are not sensitive to the cholesterol-raising effects of boiled coffee, it is difficult to make this statement with any kind of certainty, as there is a possibility that the cholesterol-raising factor in boiled coffee may be lost during the process of freeze-drying. Methodologically speaking, although the experimenters did control for bodyweight and used a standard diet, once again, the effect on females was not explored.

Results of a study using rhesus and cebus monkeys showed similar findings to the previous study.[64] Terpstra et al.[64] tested a solution of coffee bean oil containing the diterpenes cafestol and kahweol, which had been previously tested in their laboratory and shown to greatly elevate cholesterol in humans. These investigators then tested the coffee bean oil from the same batch by feeding it to male and female cebus and rhesus monkeys. Two groups of eight cebus monkeys were fed a purified diet containing 0.5% coffee oil or placebo oil (sunflower plus palm oil, 3:2, weight per weight [wt/wt]) for seven and a half weeks in a crossover design. The daily intake of the coffee oil was approximately 6-times higher than that in the human study, where strong elevations in cholesterol were seen as result of ingestion of coffee bean oil. Coffee oil did not affect a plasma cholesterol or triglyceride concentrations compared with the placebo oil. Two groups of three rhesus monkeys were fed a commercial diet containing either 0.5% coffee oil or 0.5% placebo oil for 6 weeks in a crossover design. The daily intake of coffee oil was >6-times that of human consumption shown to greatly elevate serum cholesterol and, once again, there was no effect of coffee oil on plasma cholesterol or triglyceride concentrations in monkeys. The findings from this study also suggest no effect of the cholesterol-raising diterpenes from coffee oil present in boiled coffee on cholesterol. Therefore, it is suggested that these effects may be specific for human primates, because coffee oils have been shown to consistently raise cholesterol in humans, but not in other primates.

Other research in hamsters and rats further confirms what the previous two studies found: no significant rise in cholesterol due to consumption of boiled coffee in hamsters and rats.[65] In a continuous search for an animal model for the effect of coffee lipids on serum cholesterol concentrations, these investigators fed male and female hamsters and rats diets consisting of a purified base diet and either boiled water, unfiltered boiled coffee or filtered boiled coffee. After a feeding period of 8 weeks there was no statistically significant effect of unfiltered boiled coffee on serum total cholesterol concentrations in either the hamsters or the rats. The initial level of serum cholesterol did respond predictably by rising in response to the addition of cholesterol and/or saturated fatty acids to the diet. The investigation also showed that the lack of effect of unfiltered boiled coffee in the hamsters and the rats, when compared with the previously reported activity in humans, could not be explained by dosage, duration of treatment, mode of administration or by insufficient statistical power. Therefore, this study is consistent with the previous literature[63] showing that hamsters are insensitive to unfiltered boiled coffee and therefore are not suitable models for investigating its hypercholesterolaemic effect. This study also contributes to the previous literature by showing that the same is true for rats in that they do not show the hypercholesterolaemic effect in response to coffee that humans show.

Lastly, in a review of the literature dealing with the validity of animal models for the cholesterol-raising effects of coffee diterpenes in human participants,[66] investigators searched for an animal species in which cafestol and kahweol would increase cholesterol similarly to humans (by greatly increasing LDL-C concentration). In the primate species, where a rise was seen, the rise in total cholesterol was less pronounced than that seen in human participants. In contrast to what is found in humans, the increase in total cholesterol was predominantly due to a rise in HDL-cholesterol rather than LDL-cholesterol. In other animal species, cafestol and kahweol did not raise cholesterol consistently. Most studies found no significant rise in cholesterol due to kahweol and cafestol. The variability in effects on serum lipids could not be explained by the mode of administration or dose of diterpenes, nor by the amount of cholesterol in the diet.[66] Therefore, when searching the animal literature for a valid model in which cafestol and kahweol elevate plasma lipoproteins to the same extent as in human participants, one could not be found.

Therefore, at least at this time, the scientific evidence leads us to believe that studies on coffee and caffeine's mechanism of action should be done in human participants.

3.5 The Effects of Coffee Oil Diterpenes on Cholesterol: Cafestol and Kahweol

Analyses of the substances present in coffee oil responsible for raising cholesterol levels led to two components: cafestol and kahweol, classified as diterpenes, which are found in the lipid component of coffee and mostly removed through filtering. Unfiltered coffee brews contain approximately 1–2g of lipids per litre, of which approximately 10% are diterpenes.[57] The coffee diterpene, cafestol, occurs in both robusta and arabica beans, while the related compound kahweol occurs only in arabica beans.[67] Through controlled experiments, investigators were able to determine that diterpenes also are responsible for the cholesterol-raising effects of unfiltered coffee.[53,68] In a study using coffee brews containing the diterpene cafestol, increased serum triglycerides and alanine amino-transferase, as well as decreased levels of serum creatinine and γ-glutamyl transpeptidase, were observed.[68]

Urgert et al.[69] studied the effects of prolonged intake of cafeteria coffee, which is rich in the diterpenes cafestol and kahweol, versus filtered coffee, on serum amino-transferase and lipid concentrations in healthy men and women aged 19–69 years. Cafeteria coffee raised alanine amino-transferase (a liver enzyme) concentration by up to 80% above baseline values compared with filtered coffee and also raised LDL-C concentrations by 9–14%. All increases were reversible following cessation of coffee consumption. These two studies indicated that consumption of coffee diterpenes may affect liver cells as suggested by increases in serum alanine amino-transferase concentration. These results indicate that ingestion of cafestol is accompanied by alterations in liver function enzymes; however, the mechanisms underlying these changes are not fully understood.

As the previous studies[68,69] showed, coffee diterpenes appear to affect the liver, so investigators began to search for other molecules found in the liver that may be associated with a rise in cholesterol and thus an increased risk for CVD. One of these molecules is lipoprotein (a), which consists of a large glycoprotein attached to an LDL molecule.[70] The main source of apolipoprotein (a) is the liver, and indeed, serum levels of lipoprotein (a) are determined by the rate of this molecule's production in the liver.[71] Urgert et al.[72] investigated the association between intake of boiled coffee and serum levels of lipoprotein (a) in healthy male and female Norwegian people aged 40–42 years. Participants who habitually consumed ≥5 cups of boiled coffee per day were compared with filtered coffee consumers who ingested the same amount. Participants consuming ≥9 cups of coffee per day had higher lipoprotein (a) levels than those drinking 5–8 cups per day, although the elevations did not quite reach statistical significance. However, in a subsequent study of four randomised, controlled trials using healthy male and female participants, Urgert et al.[73] reported a 4% decrease in lipoprotein (a) in response to coffee diterpenes ingestion after 4 weeks. Although these findings may initially appear indicative of a protective health effect against CVD, a meta-analysis of 11 trials with coffee preparations rich in cafestol and kahweol indicated that each 10mg of cafestol ingested per day raises serum total cholesterol by 0.15 mmol/L, which was mostly due to an increase in LDL-C.[74] The hypercholesterolaemic property of coffee diterpenes therefore undermines any potential benefits brought about by a small decrease in lipoprotein (a).

In a continuous search for a mechanism explaining the effects of diterpenes on cholesterol, van Tol et al.[75] investigated whether the coffee diterpenes may affect lipoprotein metabolism via effects on cholesteryl ester transfer protein (CETP), phospholipid transfer proteins (PLTP) and lecithin : cholesterol acyltransferase (LCAT). CETP catalyses the transfer of cholesterol esters, synthesised by LCAT, from HDL to LDL and very low-density lipoprotein (VLDL).[76] PLTP can affect the net mass transfer of phospholipids between lipoproteins, and also converts small HDL molecules into larger and smaller HDL molecules.[77] Both CETP and PLTP appear to play a major role in determining the size and quantity of HDL particles in plasma.[76] Therefore, to study these proteins, the current study used a randomised, double-blind crossover design with ten healthy male volunteers. Either cafestol or a mixture of cafestol and kahweol was given to participants for 28 days. Compared with baseline values, cafestol significantly raised the activity of CETP and PLTP ($p < 0.001$), and LCAT activity was significantly reduced ($p = 0.02$). This study provides a mechanism by which coffee diterpenes could influence serum lipoprotein metabolism. For example, because CETP catalyses the transfer of cholesterol esters, synthesised by LCAT, from HDL to LDL and VLDL, the increase in CETP levels seen here may explain the rises in LDL-C in response to coffee diterpenes.

In an attempt to determine whether cafestol and kahweol had different effects on serum lipid and aminotransferases, Urgert et al.[67] designed a randomised, double-blind crossover study, where ten healthy male volunteers were given either pure cafestol or a mixture of cafestol and kahweol for 28 days. Relative to baseline values, cafestol significantly raised total serum cholesterol concentrations, LDL-C, fasting triacyglycerols and alanine amino-transferase (all $p < 0.01$). Relative to cafestol alone, the mixture of cafestol plus kahweol increased did not significantly add to the increases seen in total cholesterol ($p = 0.08$), LDL-C ($p = 0.09$) or triacylglycerols ($p = 0.20$), but did significantly increase alanine aminotransferase ($p = 0.004$). This study shows that the effect of cafestol on serum lipid concentrations was much larger than the additional effect of kahweol, which indicates that the hyperlipidaemic effects of unfiltered coffee mainly depends on the cafestol content.

As van Tol et al.[75] had previously shown, the consumption of coffee diterpenes is associated with increased serum activity levels of the lipid transfer proteins CETP and PLTP, as well as decreased activity of LCAT. Due to these findings, the investigators designed a study examining the long-term effects of French-press coffee (rich in diterpenes) on serum lipid transfer proteins and LCAT. Additionally, these investigators compared the initial changes in CEPT activity with the initial changes in LDL and HDL-C as well as triglycerides.[78] Forty-six healthy participants ingested 0.9L per day of either French-press or filtered coffee for 24 weeks. Compared with baseline levels, French-press coffee significantly increased average CETP activity by 18% after 12 weeks, and by 9% after 24 weeks. PLTP activity was significantly increased by 10% after 12 and 24 weeks. LCAT activity was significantly decreased by 6% after 12 weeks and by 7% after 24 weeks. The increase in CETP preceded the increase in LDL-C, but not the increase in total triglycerides, demonstrating that the consumption of coffee diterpenes causes a long-term increase in CETP as well as PLTP activity; the increase in CETP activity may contribute to the rise in LDL-C.

3.6 Additional Biobehavioural Factors that May Contribute to the Effects of Caffeine on Cholesterol and Cardiovascular Disease

The review of the literature indicates direct effects of coffee preparation and caffeine on changes in lipid markers of CVD risk. However, it is important to understand the role that biobehavioural factors may play in the caffeine-CVD risk relationship because they can moderate the effects that changes in blood markers can have on health and disease outcomes. For example, individuals who have a family history of hypertension (i.e. parent is diagnosed with hypertension) appear to be particularly sensitive to the blood pressure effects of caffeine.[79] Sections 3.6.1–3.6.3 focus on biobehavioural factors that may influence the cardiovascular health outcomes of caffeine and coffee.

3.6.1 Genetic Influences

Although genetic factors play a role in the development of CVD, we now know that genotype alone does not singly determine whether an individual will develop a given health disorder.[80] The only study to date that we have found that examines a genetic component of cholesterol responses to caffeine recently was by Boekschoten et al.[81] These investigators noticed that both animals and humans show some consistency in the response of their serum lipids to fat-modified diets, which may be indicative of a genetic basis underlying this response. The investigators sought out to determine to what extent the effect of coffee oil on serum lipid concentrations was reproducible within participants. The serum lipid response of 32 healthy volunteers was measured twice in two separate 5-week periods in which coffee oil was administered (69mg cafestol/day). Total cholesterol levels increased by 24% in period 1 and 18% in period 2, LDL-C by 29% and 20%, triglycerides by 66% and 58%, while HDL-C did not change significantly. An increase in total and LDL-C of even 20% may significantly increase one's risk for CVD. For example, for an individual with total cholesterol levels of 170 mg/dL, a 20% increase means an increase of 0.97 mmol/L, elevating his/her cholesterol to 5.3 mmol/L, and almost doubling his/her chances of developing CVD. The correlation between the two responses was 0.20 for total cholesterol, 0.16 for LDL, 0.67 for HDL and 0.77 for triglycerides. Therefore, the responses of total and LDL-C to coffee oil were poorly reproducible within subjects. However, the responses of HDL and triglycerides appeared to be highly reproducible, which is suggestive of a genetic basis. It appears, therefore, that examining the genetic sources of the variation in the serum-lipid response to coffee oil is more promising for determining HDL and triglyceride levels.

An important limitation of this study is that although cafestol is known to potently increase serum lipid levels, scientists do not know at this time whether this oil is a good candidate for the study of variation in genes regulating the serum-lipid response. Additionally, participants in this study did not follow a controlled diet. Therefore, the response seen in serum lipids to coffee oil may have been due to other dietary factors not accounted for in the study. While this study shows a promising area to be explored, at least with respect to changes in HDL and triglycerides in response to coffee oil, this is the first study to date reporting on potential genetic underlying factors related to a coffee/serum lipid connection. Other studies are certainly needed that would continue to examine these variables, as well as use other populations that include non-healthy participants, and make use of better controlled dietary intake.

With respect to race differences in caffeine metabolism, there have been no published data indicating race differences in the metabolism of caffeine, although a 1999 report on food group behaviour of a mixed ethnic population did report that White elderly participants consumed more caffeine than elderly African American participants.[82] Because this study did not measure caffeine metabolism, it is difficult to say whether their findings of greater consumption of coffee by elderly White participants compared with African Americans is due to genetic differences in the metabolism of caffeine.

Another important genetic component that needs to be further explored with respect to the connection between coffee, caffeine, and CVD is family history of hypertension. Family history of hypertension illustrates the importance of genetic influence on future development of CVD. Different studies have found that normotensive offspring of hypertensive parents are at a significantly higher risk for developing essential hypertension[83,84] and increased left ventricular mass (LVM).[83,85] Children with a positive family history of hypertension show higher systolic blood pressure (SBP) levels than those children with a negative family history for hypertension.[83,86] Similar findings have been observed in adolescents[83,86] and young adults,[84] with adolescents and young adults with a family history of hypertension displaying higher SBP than those without a family history of hypertension. In addition, a higher LVM adjusted for body surface area has been observed in adolescents[83,87] and young adults[85,88] from hypertensive parents compared with those from normotensive parents. To this date, the studies that have examined the connection between caffeine and family history of hypertension have found that those participants with a family history of hypertension display higher SBP and heart rate in response to caffeine and stress than do those participants without a family history of hypertension.[79,89] No studies to date have examined whether this increased reactivity in participants with a family history of hypertension in response to stress and caffeine alters blood markers of CVD, such as CRP and fibrinogen.

3.6.2 Sex Differences

The only study to date that specifically aimed at examining sex differences in the response of serum cholesterol to unfiltered coffee was published in 1999. This study was designed to study the effects of dietary changes, which included responses to the coffee diterpene cafestol, in nine trials including 133 participants (72 men and 61 women).[90] All participants included in this study were lean and healthy. The reduction of total cholesterol in response to a decrease in the intake of cafestol was significantly larger in men than in women. However, a study conducted by el Shabrawy Ali and Felimban[91] shows that in a trial of 252 adult drinkers or non-drinkers of Arabic coffee, both male and female coffee drinkers showed significantly higher levels of total cholesterol than non-coffee drinkers, but this rise was significantly greater in women compared with men. These results indicate that cafestol is a greater risk factor for rises in cholesterol in women compared with men. These findings are particularly troubling considering that a number of studies have not included women in their sample population, or have not separately analysed data from men and women.[49,50,72,92] More studies including both men and women in their sample population are clearly needed.

3.6.3 Tobacco Smoking

Early studies on the connection between coffee consumption and tobacco smoking indicate that tobacco smoking increases the speed of caffeine metabolism,[93] and that caffeine consumption is positively associated with cigarette smoking.[94] Therefore, it is important to determine whether smoking in the presence of coffee leads to more of an increase in cholesterol levels through mechanisms other than increased coffee consumption. Furthermore, a previous epidemiological study found a significant positive linear trend between coffee consumption and total serum cholesterol that was only seen in smokers.[49] In 1993, Mensink et al.[95] examined the relationship between coffee consumption and serum cholesterol while taking smoking history into account. A large German population sample of 6820 men and 7258 women was used to investigate the relationship between coffee consumption, total serum cholesterol and HDL-C. Analyses were conducted on men and women separately. This study controlled for a number of variables, such as: BMI; diastolic blood pressure; smoking habits; alcohol, fish, milk and tea consumption; physical activity; and medication use. For men, a positive relationship between coffee consumption and total serum cholesterol was found among smokers and life-long abstainers. Interestingly, however, this positive relationship was not found in the group of ex-smokers. The lack of relationship between coffee consumption and cholesterol elevations in the group of non-smokers is not understood. In women, a relationship between coffee consumption and total serum cholesterol was also present, but very weak. Similar results were seen from previously described findings of the Olivetti Heart Study,[49] demonstrating a positive relationship between serum cholesterol levels and coffee consumption in smokers. However, the Olivetti Heart Study did not include women, therefore, comparisons between these two studies with respect to sex cannot be made. At the current time, studies examining the effects of smoking on the coffee/cholesterol connection are scarce, and more studies in this area are clearly needed. It is also important to note that, as indicated in previous studies, smoking does appear to speed up caffeine metabolism, therefore, it is important that smoking status be controlled when studying the relationship between caffeine and cholesterol.

4. Caffeine and Coffee Effects on Fibrinogen

At this time, only three studies have examined the effects of coffee or caffeine on fibrinogen.[96-98] The findings are conflicting, with some investigators finding that coffee consumption was associated with increased fibrinogen levels,[96] while others found no increase in plasma fibrinogen levels in response to coffee or caffeine consumption.[97,98] For example, Happonen et al.[96] reported that in a large cross-sectional study, middle-aged men who consumed >4 cups of coffee per day had significantly higher fibrinogen levels than did those who did not drink coffee. Conversely, Bak and Grobbee[99] found no effect of coffee or caffeine consumption on fibrinogen plasma levels among young, healthy, men and women in two randomised trials. In the first trial, 107 participants either drank filtered coffee, boiled coffee, or no coffee for 9 weeks. In the second trial, 69 participants either received 4–6 tablets of 75mg of caffeine, or the same amount of placebo tablets. No significant differences were seen between baseline levels of fibrinogen and levels obtained after 9 weeks of either boiled coffee, filtered coffee, or caffeine consumption. Similarly, Naismith et al.[98] reported no change in fibrinogen levels among 14 participants aged 21–49 years (regular coffee drinkers) in response to coffee abstinence for 2 weeks.

Due to great differences in the methodology of these studies, comparisons between these findings are difficult to make. For example, Bak and Grobbee[99] used a sample of young and healthy participants, while Happonen et al.[96] used a sample of middle-aged participants. It is possible that increasing age may be a factor in the response of fibrinogen to coffee and caffeine, therefore, this may explain why increased levels in response to coffee were only seen in the study of Happonen et al.[96] but not the study of Bak and Grobbee.[99] Although Naismith et al.[98] included a broader age range, this study had such a small sample size (n = 14) that significant changes in fibrinogen levels may not have been captured. Overall, very little data are currently available, making definite conclusions difficult at this point. More studies in this area are certainly needed, especially those examining coffee and caffeine in quantities that are similar to those commonly consumed by millions of people, and carefully controlling for behaviours that can influence fibrinogen such as tobacco smoking and dietary habits.

5. Caffeine, Coffee and CRP Levels

To this date, there are no published reports examining the relationship between coffee or caffeine by itself on levels of CRP *in vivo*, and only one study exploring this relationship *in vitro*.[100] In the only study reported to date, the investigators evaluated the effects of caffeine on acute-phase proteins (CRP being one of these proteins). Changes in the concentration of a group of plasma proteins called acute-phase proteins represent an important biochemical response to tissue injury or infection.[101] Within the first few hours after an inflammatory stimulus, the hepatic rate of synthesis of a number of plasma proteins increases, while that of some others decrease. CRP is one of the two major acute-phase proteins that increase in plasma concentrations as much as several-thousand-fold.[100] The synthesis of acute-phase proteins is regulated by cytokines, and this study explored the signal-transduction mechanisms by which cytokines regulate the synthesis of acute-phase proteins in human hepatoma cells and how this process is affected in the presence of caffeine. Two mmol/L of caffeine led to a significant potentiation of CRP induction, ranging from a 40- to 180-fold increase above that seen in control cultures. Although the exact mechanisms by which caffeine exerts its biological effects are not fully understood, they are believed to be mediated through increases in intracellular concentrations of cyclic adenosine monophosphate or calcium.[100] In a different study, 100mg of caffeine (approximately 1 cup of coffee), led to a highly significant increase of 150% on platelet reactivity.[102] More research is still needed to elucidate the effects of caffeine on CRP levels; however, it is known that platelets contribute to CVD by, among other mechanisms, stimulating inflammation,[103] and it also known that CRP is an immune marker of inflammation.[33-36] If future research finds that there is indeed a positive relationship between caffeine consumption and CRP, then caffeine's increase in platelet reactivity may serve as a potential mechanism that would help to explain the relationship between CRP and caffeine.

Although an increase in CRP was seen in hepatoma cells in the presence of caffeine, it is nearly impossible to predict whether similar findings would occur in the plasma levels of living human populations as a response to caffeine. Only further studies in this area will help to elucidate this relationship.

6. Conclusions

Boiled, unfiltered coffee consumption may increase the risk of developing CVD as indexed by changes in lipoproteins (i.e. cholesterol, triglycerides). The cause of this cholesterol increase seems to be the ingestion of coffee diterpenes, which are removed through filtering. The effects of boiled or filtered coffee on fibrinogen levels are equivocal probably because of the paucity of data collected in this area. One study suggests that coffee consumption does increase fibrinogen, but the coffee preparation methods were not reported. Studies that did control for coffee preparation methods reported no effect of boiled, unfiltered coffee on fibrinogen levels, but this lack of an effect could be the result of large differences in the age of participants among studies, as well as health status or lack of control over basal cholesterol levels. Further studies clearly are needed in this area before any definite conclusions can be drawn regarding the health effects of coffee – boiled or not – on fibrinogen levels in men and women. This final statement also can be made with regard to CRP in that there are no published reports on the influence that coffee intake can have on CRP levels. This is a critical gap in the research area given the independent role that CRP may play in the development of CVD.

With regard to the effects of caffeine on CVD risk markers, the research findings are far scarcer in determining the causal relationship between caffeine alone and blood markers of CVD risk. Cholesterol levels do not appear to change in response to caffeine consumption among healthy adults. Observed cholesterol changes in response to caffeine administration seem to be influenced by biobehavioural factors such as stress, smoking status, lipid status, age, diet and family history of hypertension. More specifically, some studies do not detect a measurable cholesterol change to caffeine consumption, but this could be a result of inadequate or incomplete dietary histories, difficulty with statistical analyses due to inappropriate sample size, failure to adjust for covariates that may have been confounders, lack of non-healthy populations, inclusion of elderly participants whose serum cholesterol levels are markedly higher than a younger cohort, daily/personal stress levels, and failure to include women as well as minority populations. Overall, the data suggest that individuals already at risk for developing CVD (e.g. elevated cholesterol or blood pressure, family history of hypertension) should avoid high levels of coffee intake, particularly if the coffee is unfiltered or boiled. Recommendations for healthy men and women are difficult to make at this time until further, better controlled studies are done.

With respect to fibrinogen, one study reported no effects of low-dose daily caffeine consumption in the form of a tablet.[21,45,52,53,59,104] Given that this is the only published report that we could find in this area, a consistent effect of caffeine on fibrinogen remains elusive. Caffeine effects on CRP in humans have not been reported to the best of our knowledge, but *in vitro* results do suggest a direct potentiating effect of caffeine on CRP induction from the liver,[21,45,52,53,59,104] the result of which would be elevated CRP levels *in vivo*. Given the small number of published reports, it is difficult to make any definitive conclusions about the effects of caffeine on fibrinogen and CRP levels in humans. There clearly is a need for further laboratory, controlled studies as well as epidemiological explorations of these caffeine-CVD relationships.

In evaluating the statistical analyses used by the authors cited in this review, several main issues become apparent and need to be considered in future investigations. First, in some instances, sample sizes were either so large[28,50,105,106] or so small[50,53,72,75,104,105] that statistical evaluation of the clinical outcome was difficult and potentially misleading. Very large sample sizes may lead to statistically significant effects that may not be clinically relevant. Conversely, the randomised clinical trial conducted by Forde et al.[104] was composed of a total of 33 participants divided into four groups, with eight or nine participants in each group. While the randomised clinical trial is a strong design, the opportunity for bias in using such a small sample is self-evident. Findings from such studies provide much less generalisability and little opportunity (due to less power) to find significant relationships that may exist. Second, the vast majority of studies used multivariate statistical analyses to consider covariates and to assess any additional relationships that may have occurred. Given the complexity of choices involved in such analyses, it is extremely difficult to comment on the appropriateness of the choice of statistical models used. If multiple variables are believed to be involved, the choice of multivariate analyses appears to be appropriate. However, a number of studies neglected to analyse any factors as covariates.[52-54,98] Third, it is important to report overall caffeine intake as indexed by coffee consumption for between-study comparisons. More specifically, it was difficult to assess the actual amount of coffee consumed by participants across the studies in the present review, as some studies[21,45,52,59,98,104] neglected to report coffee cup size.

For several reasons, it also is important to consider the health status, age, sex and ethnic status of participants in future investigations. To date, only a few studies examined the effects of diterpenes on non-healthy (hypercholesterolaemic) populations.[45,55] It is understandable that investigators may want to exclude participants falling into this category because it is still uncertain whether these participants respond to changes in coffee diterpenes in the same way that healthy participants respond (e.g. hypercholesterolaemic participants may be hyper- or hypo-responders). However, this is an important population to be studied because they appear to be at greater risk for CVD and may, therefore, benefit even more from this information than will healthy participants. With regard to age, the Lipids Research Clinics Program made an extensive survey of the distribution of lipoproteins in the American population as a function of age and found that cholesterol levels rise substantially with aging. For example, total cholesterol concentrations of 3.9 mmol/L in a 20-year-old individual are comparable with 5.2 mmol/L in a 50-year-old person. As a result, lack of even age distribution across studies may affect observed cholesterol changes in a particular study. Finally, elderly participants metabolise coffee more rapidly than those aged <65 years,[107] and may respond differently to coffee consumption than do younger participants. With regard to sex, caffeine metabolism among women also changes throughout the menstrual cycle (e.g. 25% decrease in caffeine elimination during the luteal phase[28,33-36]), which may also be a source of bias across studies. Further investigations that control for these variables are very much needed to help the field move forward.

The issue surrounding sex is not inconsequential: a common misconception with respect to CVD is that men experience this condition more than women. However, according to the American Heart Association,[108] in terms of total deaths, in every year since 1984, CVD has claimed the lives of more women than men. Furthermore, the gap between male and female deaths has increased dramatically over the years. These staggering statistics stress the importance of including women in studies investigating the connections between blood markers, coffee and caffeine, and risk factors for CVD.

Last, a very discouraging observation is that none of the reviewed studies reported any attempts to investigate the potential effects of race on the relationship between coffee and caffeine consumption and increased risk for CVD. The American Heart Association[108] has reported that the overall death rate in 2000 from CVD was significantly greater among Black men versus White men and also significantly greater among Black women compared with White women. Furthermore, Black and Mexican-American women have higher cardiovascular risk factors than White women of comparable socioeconomic status.[108] Therefore, there is a great need for studies that include minority populations in their examination of risk factors for CVD in respect to coffee and caffeine.

According to the American Heart Association,[108] heart disease continues to be the number one killer in America, and claims more lives each year than the next five leading causes of death combined. Therefore, it is extremely important to conduct research that will lead to further understanding of this field and, perhaps, contribute to a decrease in the number of deaths brought about by CVD. Studies examining blood markers for CVD such as CRP and fibrinogen in relationship to coffee and caffeine would certainly advance this area.

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